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TI Pyrophosphorylation by Type II DNA polymerases: implications for pyrophosphorylation-activated polymerization.
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AB We find that Type II DNA polymerases can catalyze pyrophosphorylation, the reverse reaction of DNA polymerization. This property is applied utilizing pyrophosphorylation-activated polymerization (PAP), a method of nucleic acid amplification using serial coupling of pyrophosphorylation and polymerization. PAP can be used for ultrarare allele detection (detection of minimal residual disease and cancer risk assessment through measurement of mutation load) and for microarray-based scanning for unknown mutations. Herein, we show that Type II DNA polymerases efficiently catalyze template-dependent pyrophosphorylation to activate oligonucleotides blocked at their 3' termini with acyclonucleotides in which a 2-hydroxyethoxymethyl group substitutes for the 2'-deoxyribofuranosyl sugar. Type II archaeon DNA polymerases Vent (exo-) and Pfu (exo-) can be utilized for PAP or a bidirectional form of PAP with acyclonucleotide-blocked oligonucleotides, but not with dideoxynucleotide-blocked oligonucleotides. In contrast, a Type I DNA polymerase, TaqFS, can utilize either acyclonucleotide-blocked or dideoxynucleotide-blocked oligonucleotides. These findings expand the potential of nascent PAP technology.